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NOTES

Morphogenesis of poliovirus III.

Formation of Provirion in Cell-Free Extracts

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Formation of an apparent virion precursor, the provirion, can be demonstrated in cytoplasmic extracts of poliovirus-infected HeLa cells.

We have reported the isolation and characterization of a new subviral particle from poliovirus-infected cells (1). This ribonucleoprotein complex has been termed the provirion, in recognition of its apparent status as a direct virion precursor. Provirion has the protein composition of the procapsid and RNA of the same size as poliovirion RNA (35*S*).

To investigate further the mechanism of provirion synthesis and the possible role of the particle as an intermediate in virion morphogenesis, a cell-free system has been developed which allows in vitro formation of provirion. A cytoplasmic extract was prepared from HeLa cells which had been infected with poliovirus for 3.5 h and which had been labeled with ^{3}H-leucine for 30 min and with ^{14}C-uridine for 15 min. One half of the cytoplasmic extract was incubated at 37 C for 30 min, whereas the other half remained at 0 C. The extracts were then adjusted to 1% DOC and 1% Brij-58 and the samples were sedimented through sucrose gradients. The gradients were fractionated and portions of each fraction were assayed directly for acid-precipitable radioactivity (Fig. 1a and c), while equal portions were assayed after ribonuclease digestion (Fig. 1b and d).

As shown previously (1), the unincubated sample (Fig. 1a) contained virions (155*S*) and a small peak of provirions (125*S*) both of which were evident in the A_{260} profile and were labeled with both uridine and leucine. Also evident was leucine-labeled procapsid and uridine-labeled viral ribonucleoprotein. Treatment of the fractions with ribonuclease had no effect on the provirions or virions (Fig. 1b).

After incubation at 37 C for 30 min a number of effects were evident (Fig. 1c). The major difference was a marked rise in both uridine and leucine label in the region of the provirion. Most of the apparent provirion formation observed occurred during the first 15 min of incubation (results not shown). Changes in the A_{260} pattern were evident; some of these we cannot interpret but the increased A_{260} in the provirion region is evident. Whether or not this increased A_{260} is due entirely to provirion or is due partly to a cosedimenting structure is unclear. Another effect of incubation at 37 C was an increase in leucine radioactivity in the procapsid region, confirming previous results of Phillips and coworkers (2, 3). The 37 C incubation also increased the uridine radioactivity in structures sedimenting slower than 100*S*—these are presumably breakdown products of the polyribosomes and replication complex.

In order to determine if the increased radioactivity in the provirion (125*S*) region of the sucrose gradient after incubation was actually due to provirion formation, the properties of the material in that region were studied. When the fractions of the sucrose gradient were treated with ribonuclease, the uridine label in the 125*S* region was not digested (Fig. 1d). Ribonuclease-resistance is a property of the provirion (1). Analysis of the RNA and protein of the in vitro product revealed only 35*S* RNA and procapsid proteins (not shown) which are the known components of the provirion. Treatment of the 125*S* particle with EDTA disrupted it and generated protein sedimenting at the rate of procapsid (not shown) which is the behavior previously found for the provirion (1). Therefore, by all criteria, the 125*S* material which formed after incubation was indistinguishable from the provirion.

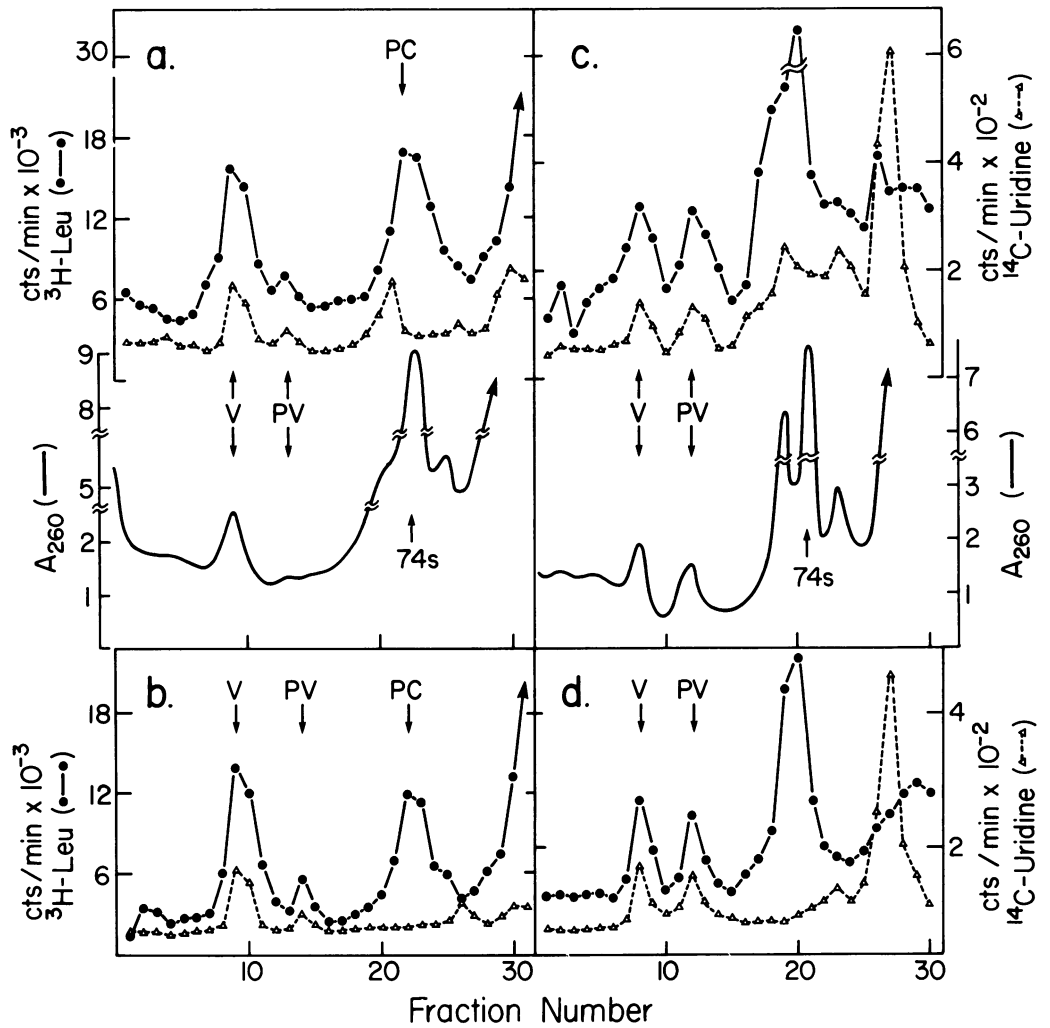

FIG. 1. *Formation of provirion in cell-free extracts. At 3 h after infection with poliovirus, type 1, in the presence of 5 μg of actinomycin D per ml, 1.6 × 10⁸ HeLa cells were labeled with ³H-leucine (20 μCi/ml) for 30 min and ¹⁴C-uridine (0.5 μCi/ml) for the last 15 min and at 3 h 30 min a cell extract in 2 ml of RSB (0.01 M NaCl, 0.10015 M $MgCl_2$, 0.01 M Tris, pH 7.4) was made as described previously (1). One milliliter of the sample was brought to 1% sodium deoxycholate and 1% Brij-58 and it was kept at 0 C; the other half of the sample was placed at 37 C and 30 min later was made 1% sodium deoxycholate-1% Brij-58. The samples were layered on 15 to 30% sucrose-RSB gradients and centrifuged at 95,000 × g for 4 h at 4 C. Absorbance was monitored at 260 nm and fractions of 1 ml were collected. Half of the volume of each fraction was precipitated with 1 volume of 25% trichloroacetic acid and radioactivity was measured; the other 0.5 ml of each fraction was made 0.3 M NaCl, 0.03 M Na citrate, and exposed to pancreatic ribonuclease A (10 μg/ml) for 5 min at 37 C before precipitation. a, Unincubated sample; b, unincubated sample treated with ribonuclease; c, incubated sample; d, incubated sample treated with ribonuclease. Abbreviations: V, virion; PV, provirion; PC, procapsid.*

The above results indicate that there is formation of provirion in a cell-free extract of poliovirus-infected cells incubated at 37 C. Neither synthesis of viral RNA nor of viral protein has been detected under the conditions of incubation of the extracts which have been used in the present work. Since provirion consists of virus-specific proteins and RNA, these macromolecules must therefore be present in some functional precursor pool in the infected cell and they must be able to aggregate into the provirion particle after the disruption of cellular integrity. The nature of the precursor pool is under study. Preliminary experiments indicate

that 2 mM guanidine hydrochloride does not block in vitro provirion formation.

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